Together with Cecil Cooper and other colleagues, Lehninger also pursued a project of "making morphologically less organized preparations from rat liver mitochondria with which the enzymatic details of oxidative phosphorylation could be more directly studied, with the ultimate goal of resolving the mechanisms of oxidative phosphorylation by enzyme separation and reconstruction approaches" (Lehninger et al., 1958, p. 450). The last clause indicates Lehninger's goal of establishing the mechanisms of oxidative phosphorylation using the traditional biochemical approaches of isolating responsible enzymes and then putting them together again into a system that performs the reaction.

The strategy Lehninger and his collaborators employed for decomposing the mechanism was to treat isolated mitochondria with digitonin to gently break down membrane lipids, which they reported caused the "virtual dissolution" of the mitochondrion, "leaving a turbid brown solution" (p. 450). They subjected this solution to centrifugation at 50,000 *g* for 25 minutes, removed the supernatant fluid, which contained a gelatinous material, and centrifuged it at 100,000 *g* for another 25 minutes. This yielded "phosphorylating membrane fragments" (p. 450), which did not catalyze most of the reactions of the citric acid cycle and generated ATP only when D-β-hydroxybutyrate or succinate (but not pyruvic acid or some other citric acid cycle intermediate) was supplied. Electron transport was evidenced by oxygen uptake, and oxidative phosphorylation by ATP synthesis (using radioactive phosphate as a tracer). Although the efficiency of the reactions was less than for intact mitochondria, they claimed that ATP synthesis did occur at all three sites along the electron transport chain. Most of the typical chemical agents that decoupled respiration from phosphorylation, such as 2,4-dinotrophenol, had the same effect on the particles, but Ca^{2+} and thyroxine did not. Appealing to electron micrographs, Lehninger and colleagues identified the particles as arising largely from the cristae.

From two facts – that the citric acid cycle enzymes could be separated in solution and that the membrane fragments isolated by Lehninger did not catalyze the reactions of that cycle – investigators could conclude that the citric acid cycle occurred in the matrix of the mitochondrion while electron transport and oxidative phosphorylation occurred in the cristae of the inner mitochondrial membrane, as Palade had suggested. The earlier determination that glycolysis occurred in the cytosol permitted localizing the three major biochemical mechanisms of cellular respiration (as detailed in Figure 3.16) in three different parts of the cell, as illustrated in Figure 6.4.

From the fact that sonic vibrations prior to centrifugation greatly reduced the sedimentation rate but not the efficacy of the particles, Lehninger and Cooper concluded that the inner membrane was comprised of repetitions